Planar Laser Imaging of Scattering and Fluorescence of Zooplankton Feeding in Layers of Phytoplankton *in situ*

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LONG-TERM GOALS

We intend to quantify the biological, physical, and chemical dynamics that structure marine planktonic ecosystems. Observations of the organisms and their environment on the spatial and temporal scales that characterize their interactions, combined with models of the dominant dynamics, will lead to improved understanding of the dynamics, structure, and function of planktonic ecosystems.

OBJECTIVES

Our objectives in this work are to 1) visualize and quantify herbivorous copepod feeding in the laboratory, and 2) to apply these methods in the field to observe the dynamics of copepod feeding *in situ*. In particular we intend to test the "feeding sorties" hypothesis vs. the "*in situ* feeding" hypothesis regarding the location and timing of copepod feeding and vertical migration.

APPROACH

Previous attempts to quantify copepod feeding have either been indirect (measuring the phytoplankton concentration before and after copepods were introduced to a sample), or direct (measuring the gut fluorescence of individual copepods feeding on phytoplankton). The disadvantage of the first method is that we obtain little information about the activities of individual copepods, and how their feeding might change in time. The second method is destructive, and generates only one data point per individual copepod, rendering it ineffective for generating time series of feeding activity. To obviate these problems, we use a planar laser imaging fluorometer (PLIF) system for quantifying copepod gut fluorescence and feeding. A green (532 nm) laser is used to stimulate the fluorescence of chlorophyll *a* ingested by copepods. The fluoresced red (685 nm) light is imaged by a very sensitive CCD camera. A second bi-spectral PLIF system images both the fluorescence from chlorophyll *a*, and the green light scattered from particles (including copepods) in the imaging plane. This method is non-destructive, allowing time-series measurements to be made on individual copepods.

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Our tasks for the second year of our research were to:

1) Run a set of feeding experiments to quantify copepod intra- and inter-individual variability, and evaluate how temperature affects individual copepod feeding behavior.

2) Run pulse-chase experiments to evaluate the traditional assumption of constancy of gut clearance in copepods that stop feeding, an assumption critical to data collected in previous studies.

3) Conduct a one-week cruise in Dabob Bay, WA to deploy a modified bi-spectral version of the FIDO-ϕ to study copepod feeding *in situ*.

4) Continue analysis of data from the temperature and pulse-chase experiments; begin analysis of cruise data.

5) Begin writing papers.

WORK COMPLETED

We have had great success in achieving our goals in this second part of our research. The experiments performed in the lab generated a large set of unique data documenting the gut activity of individual copepods. Using this data set, we are currently developing new insights into how a copepod's gut works and how that might relate to its feeding behavior. A manuscript describing the methodology used in the lab experiments is ready to submit to Limnology and Oceanography Methods.

To simultaneously image the scattering and fluorescence from copepods *in situ*, we designed a two-camera system with both cameras imaging the same plane. One camera was equipped with a 530 nm filter that would allow imaging of particle shapes using scattered light; the second camera was equipped with a 685 nm filter to image chlorophyll *a* fluorescence. The constraints imposed by the system's geometry limited our image resolution to about 80 x 80 microns in an imaging plane of 10 x 13 cm. This gives sufficient resolution to identify the species of copepod, isolate the region of the gut that is fluorescing, and quantify the intensity of fluorescence. Repeated profiling (one profile every 3-5 min from the surface down to 30 m over 2-3 h periods) of the FIDO-ϕ with the bi-spectral imaging system allowed identification of the copepods, the depth at which they were located, and whether they had recently fed on phytoplankton.

We deployed the FIDO-ϕ bispectral imaging system from the R/V Thompson in Dabob Bay in early May 2007, in conjunction with Bruce Frost and Andrew Leising's sampling work from the R/V Barnes. Our images, in combination with the Frost/Leising data and our ancillary measurements, will allow us to test the "feeding sorties" hypothesis against the "*in situ* feeding" hypothesis.

RESULTS

The objective of our first experiments was to assess how temperature affected an individual copepod's feeding behavior, and to calculate an individual Q_{10} of gut activity based on the rate of defecation. The individual variability of Q_{10} is an indication of the range of feeding behavior that might be found in the field. Until now estimates of Q_{10} were averages from 10's or 100's of animals feeding simultaneously in batch cultures. Our imaging technique allows us to estimate the Q_{10} of an individual copepod under

a range of conditions (in this case, temperature). We imaged the feeding of the same tethered copepod at 3 different temperatures, and repeated this process for several copepods. Our preliminary analyses suggest an average Q_{10} of 2.5, consistent with the literature; however, the Q_{10} for different individuals appears to vary from 1.6 to 4.7, corresponding to a wide range of feeding rates and behaviors.

Gut clearance rate has played an important role in quantifying copepod feeding dynamics: if it is in equilibrium with recent ingestion rate, measurements of gut clearance would give estimates of ingestion rate. Because gut clearance measurements require incubating copepods in filtered sea water, a necessary underlying assumption is that gut clearance remains constant when copepods stop feeding.

We conducted a set of bolus chase experiments to test this assumption. A tethered copepod was imaged while it fed. After some time, the food source was interrupted and imaging continued. The decay of the fluorescence signal in the gut was recorded. Our early results suggest that interruption in feeding changes the rate at which food is processed (Fig. 1). The constant gut clearance assumption may therefore be invalid. If true, this would mean that ingestion rates have been underestimated since the method was first applied to copepods 30 years ago. Our initial finding also suggests that the change in the gut clearance rate may be regular (Fig. 1); thus measurements made using the gut clearance method might be corrected to take into account this change in gut evacuation time.

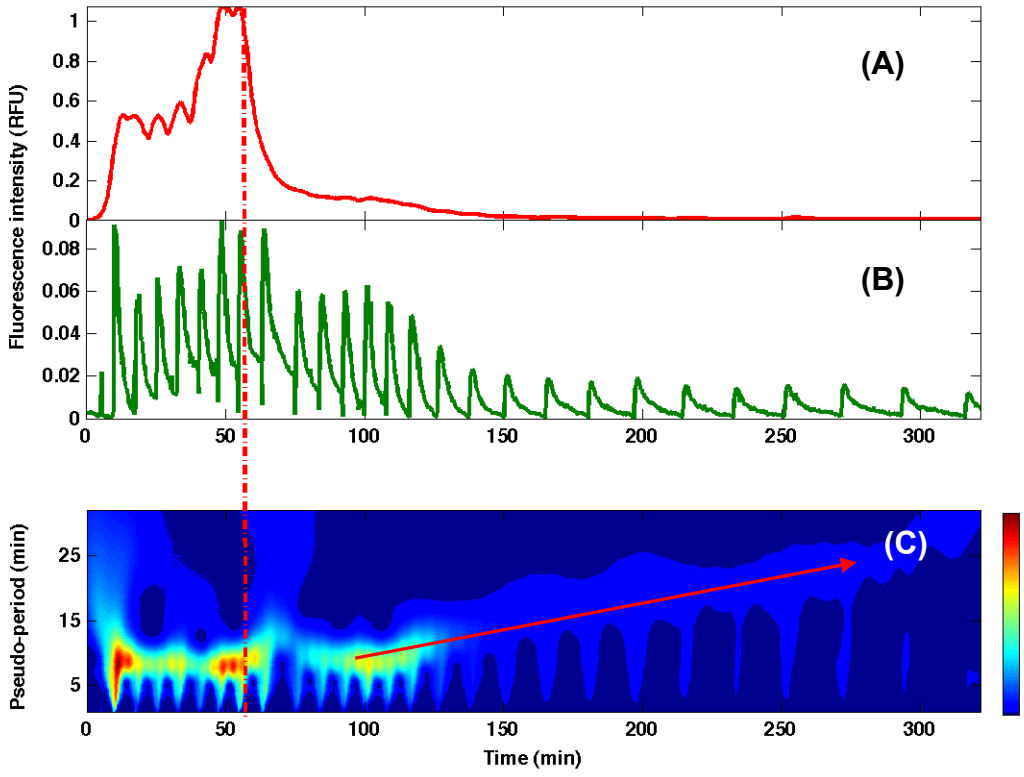

Fig 1. Data from a bolus chase experiment. Red dashed line shows time of food cut-off. (A) Upper mid-gut content, smoothed for clarity. (B) Posterior mid-gut content, where material transferred from upper mid-gut (peaks) is packaged before evacuation (troughs). Note the decrease in peak height with time (proxy for pellet size), and the progressive lengthening of evacuation interval. (C) Wavelet analysis of the posterior mid-gut signal (B) shows the regular increase in the defecation intervals (red arrow) after the food source is removed.

During our cruise in Dabob Bay, Our imaging system was deployed 3 times a night: around dusk to intercept the upward vertical migration of copepods; during the middle of the night to measure the variability of feeding status, and around dawn to intercept the downward migration. In addition to the imaging system, our sampling package included CTDs, a 1200 kHz ADCP and a SCAMP microstructure sensor. Additional shipboard CTD/rosette measurements were taken before each deployment. An example of the hydrographic data is shown in Fig 2. After a few trial deployments and fine-tuning of the system, we opted for profiles between 0 and 30 m where we expected most of the grazing to take place, thus maximizing our chances of imaging copepods.

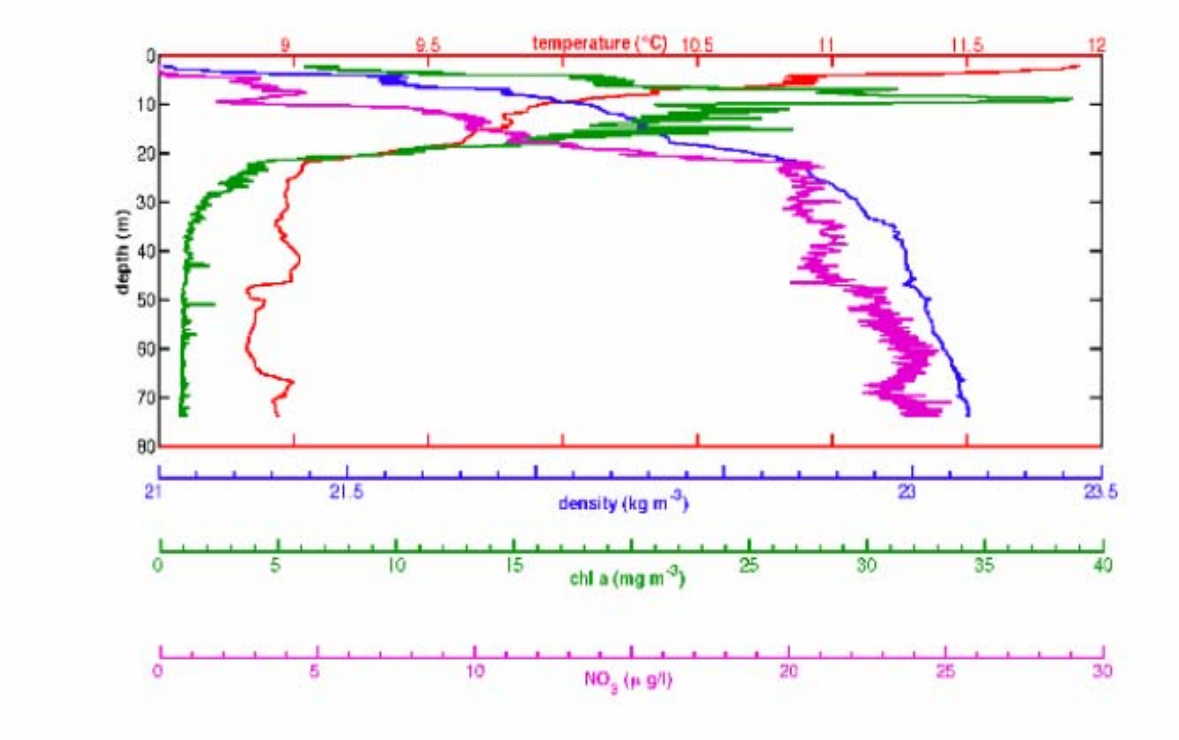

Fig 2. Hydrographic data collected with one of the CTDs mounted on the imaging package. Note the relatively shallow depth (~9 m) of the intense chlorophyll maximum. Using data such as these helped us narrow our profiling depth range to where we expected copepod grazing to be most intense. Later deployments were limited to profiling between the surface and 30 m.

The bispectral imaging system appears to have worked extremely well: we are able to distinguish among the dominant copepod species in Dabob Bay, and can quantify the distribution of phytoplankton pigment in their guts (Fig. 3). Our data will allow us to determine a copepod's location and gut content, and relate this to the distribution of resources and the distribution of the populations in relation to the phase of their vertical migration. We intend to analyze the data to determine where and when copepods are feeding, and how they behave once their gut is full. We will find out whether they remain in a food patch after their gut is filled, or if they migrate downwards to digest, and then back up again to feed. These results will have important implications for understanding trophic coupling and vertical fluxes in the plankton.

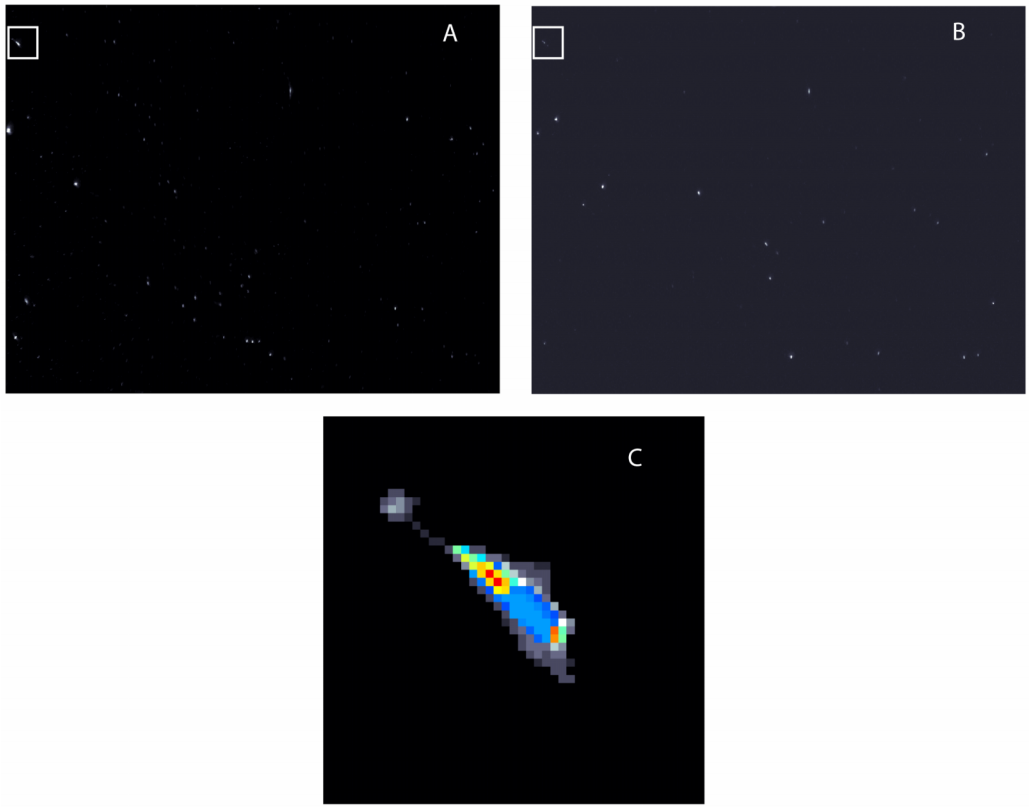

Fig. 3. In situ images from the bispectral planar laser imaging system. (A) Scattered light image. (B) Fluorescence image of the same particles as (A). Images are 10x13 cm with 80 micron resolution. In (A) and (B) a box is drawn around a copepod,* Metridia pacifica*, which is shown magnified in (C). In panel (C) the fluorescence intensity (color scale) is overlaid on the scattering intensity (gray scale) to show the parts of the copepod that contain chlorophyll* a*. The copepod is oriented prosome-down at a 45° angle, with its caudal rami toward the upper left. The areas corresponding to the bottom of the mid-gut and the foregut of the* Metridia *are fluorescing, suggesting an intermittence in its feeding.

IMPACT/APPLICATIONS

Our bi-spectral PLIF system gives us an entirely new way to gather data from planktonic organisms in the lab and *in situ*. Combined with appropriate auxiliary data, this system will allow us to investigate the dynamics of the planktonic ecosystem at the level of the individual plankters. The data generated will give us a unique and powerful new view into the dynamics structuring marine planktonic ecosystems.

RELATED PROJECTS

This work grew from our ONR-sponsored project entitled “Biological and Chemical Microstructure in Coastal Areas” in which we deployed a PLIF system in tandem with an optical nitrate sensor and microstructure sensor. Based on the information gathered in the present work, we will re-analyze the images acquired in our earlier cruises to attempt to identify zooplankton gut fluorescence in the images.